



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 35/74, 35/38	A1	(11) International Publication Number: WO 96/11014 (43) International Publication Date: 18 April 1996 (18.04.96)
(21) International Application Number: PCT/AU95/00664 (22) International Filing Date: 6 October 1995 (06.10.95) (30) Priority Data: PM 8648 7 October 1994 (07.10.94) AU (71) Applicant (for all designated States except US): BLACK-MORES LIMITED [AU/AU]; 23 Roseberry Street, Balgowlah, NSW 2093 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): BROWN, Richard [AU/AU]; 45 Livingstone Street, Belmont, NSW 2280 (AU). BORODY, Thomas, Julius [AU/AU]; 144 Great North Road, Five Dock, NSW 2046 (AU). (74) Agents: STEARNE, Peter, Andrew et al.; Davies Collison Cave, Level 10, 10 Barrack Street, Sydney, NSW 2000 (AU).		(81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG). Published <i>With international search report.</i>

(54) Title: TREATMENT OF BOWEL-DEPENDENT NEUROLOGICAL DISORDERS**(57) Abstract**

The invention relates to a method for the treatment and/or prophylaxis of a sleep disorder or for inducing sleep or for the treatment and/or prophylaxis of a neuropsychiatric disorder or for the treatment and/or prophylaxis of SIDS or for the treatment and/or prophylaxis of Chronic Fatigue Syndrome or children's *Cl. botulinum* poisoning in a mammal, comprising administering to said mammal an effective amount of whole live or dead enteric microorganisms or cell wall containing fragments thereof. The invention also relates to pharmaceutical compositions which can be utilised in the methods of treatment as well as methods of manufacturing these pharmaceutical compositions.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

TREATMENT OF BOWEL-DEPENDENT NEUROLOGICAL DISORDERS

Field of the Invention

- 5 This invention relates to a method for the treatment and/or prophylaxis of a sleep disorder or of inducing sleep or for the treatment and/or prophylaxis of SIDS or neuropsychiatric disorders in a mammal. The present invention also relates to pharmaceutical compositions used in the methods and processes for their manufacture.

Background of the Invention

10

- Sleep deprivation may be the result of a change in work patterns such as occurs with shift-workers or people working long hours, regular travel to different time zones or may also be due to a secondary effect of drug administration for other purposes. Sleep deprivation may also fall within the category of conventional amnesia which can occur
15 for a wide range of medical and/or psychological reasons. Sleep deprivation is not only distressing and upsetting for the patient concerned, but it is also recognised that sleep deprivation has a suppressing effect upon the immune system.

- There are many reports of a sleep-inducing substance(s) in the brain and in the body
20 fluids of animals deprived of sleep. Increases in sleep behaviour can be observed by administering extracts from sleep-deprived animals into normal waking recipients^{1,2}. A low molecular weight peptide with potent sleep-inducing properties has been isolated from the brain, cerebrospinal fluid and urine of various mammalian species including man^{1,2,3}. The peptide Factor S (FS) has specifically been shown to increase the Slow
25 Wave Sleep (SWS) component of sleep in rates and rabbits without affecting the Rapid Eye Movement (REM) component of the sleep cycle. It has also been shown that Factor S accumulates in the brain during periods of wakefulness and then declines exponentially during periods of sleep. Concurrent with this increase in the amount of slow-wave sleep, the immune system is also stimulated in patient's whose immune system had previously
30 been depressed due to sleep deprivation. The 'natural' aspects of sleep-associated behaviour are preserved in FS-treated animals, namely spontaneous waking, grooming

- 2 -

and feeding, and the cyclic nature of the sleep-wake pattern is also preserved⁴.

The structure of FS has been shown to be a muramyl peptide possibly of the sequence N-acetyl-glucosaminyl-N-acetyl-muramyl-L-alanyl-D-glutamyl-mesodiaminopimelyl-D-alanine⁵. Muramyl peptides occur naturally as monomeric components of the bacterial cell wall⁶ and to our present knowledge are unable to be synthesised by mammalian cells⁷. Recently Kreuger *et al*⁸ speculated on the possible relationship between the ontogeny of SWS and the colonisation of the gastrointestinal tract with bacteria. Stimulated by such speculation Brown *et al*⁹ proposed that the ontogeny of SWS parallels the colonisation of the gastrointestinal tract in animals by the so called autochthonous flora, and that this flora can be acquired in the lower animals such as rats, by coprophagy supplying a source of FS-like products while colonising the gastrointestinal tract with adult bowel flora.

The gastrointestinal tract of mammals is sterile before birth. During delivery and on exposure to the environment there occurs a rapid infestation or colonisation of the intestinal tract with microbes¹⁰. In the rat, for example, within the first few hours of life there appears colonisation by *Lactobacillus* and *E. coli* which remain dominant until the establishment of the strict anaerobes by the third week of life. The strict anaerobes comprise as much as 99% of the gut flora^{10,11}. *Bacteroides* species are amongst the most predominant of the strict anaerobes^{10,11} and, along with several less common bacterial species, make up what is collectively known as the autochthonous flora, native to the gastrointestinal tract of the host. During the colonisation process the development of the anaerobic flora is delayed by some three weeks after the onset of *Lactobacillus* colonisation, and the total numbers of the anaerobes is considerably higher. Large amounts of REMS with almost no SWS is the typical pattern shortly after birth¹². A gradual increase in SWS with concomitant decrease in REMS occurs over several weeks until adult patterns stabilise shortly after weaning¹². A lag of approximately 5 days occurs between the appearance of *Bacteroides* and the first increases in SWS. Similarly, adult *Bacteroides* levels are reached 5 days before SWS levels stabilise (see the Figure of reference 9). This would suggest that the lag time may be due to the accumulation

- 3 -

- in the host of bacterial cell wall needed to produce SWS⁹. These bacterial strains reside in almost direct contact with the intestinal wall in mucus in the crypts of the large intestine¹³ making the breakdown products of this group of microbes more readily adsorbable. Also, the immune response to *Bacteroides* sp. as demonstrated in the rodent, is almost non-existent with natural antibody titer very low when compared with, for instance that against *E. coli*¹³. Yet, in humans, systemic, urinary or wound infections are exceedingly uncommon, when compared with *E. coli*. In effect, the *Bacteroides* sp. are treated as self by the host thus avoiding the threat of immune system attack.
- 10 Further evidence of bowel flora development in relation to SWS comes from another model, the guinea pig. In this animal, unlike the rat, adult levels of SWS and REMS are evident from day 1 of post-natal life¹². It is significant to note therefore, that guinea pigs are coprophagic from birth, consuming the mothers faeces and so implying intake of enterobacterial sleep factors¹⁶. This would suggest that the microorganisms consumed
- 15 in the faeces contribute to the early appearance of adult sleep patterns. Compelling evidence for the role of muramyl peptides in the ontogeny of sleep behaviour has been reported by Davenne and Krueger¹⁷ who observed large increases in SWS and decreases in REMS in neonatal rabbits following administration of muramyl dipeptide (MDP). These researches state that a mechanism responsive to MDP already is established in
- 20 the neonate and merely awaits triggering. In addition, deliberate depletion of the autochthonous bacterial population in rats using antibiotics giving an 80% reduction in the bowel flora, resulted in significant reduction in SWS during the first three hours of the recording sessions, without effect on REM sleep¹⁸.
- 25 Other support for the involvement of gut microorganisms and hence muramyl peptides in sleep behaviour comes from the findings of Rhee and Kim¹⁹ who showed marked decreases in gastrointestinal flora in patients with insomnia. They also found decreases in sleep times of hospitalised general ward patients who were on antibiotic therapy or other antimicrobial treatments. Normal subjects given strong antibiotic regimens
- 30 displayed decreased sleeping times. These decreases in sleep times were accompanied by dramatic decreases in bacterial colony numbers. Clinical observations in the human

- 4 -

newborn child are also of value. In human neonates, initially an approximately 4 hourly sleep-feed pattern is present during breast or bottle milk feeding, supporting the predominantly *Lactobacillus infantum* bowel flora, and this later changes to the more adult pattern of sleeping through more of the night, as solids are introduced into the diet
5 presumably stimulating the growth of colonic anaerobes, again predominantly *Bacteroides* sp. It has been noted that malnourished human infants have exhibited high REMS times and relatively low SWS times when sleeping¹⁴. Subsequent nourishment inverted these levels to normal, perhaps due to the inability of the malnourished child to maintain a full complement of enteric microorganisms, hence reducing the source of
10 FS.

Indeed, dietary changes in mice have produced dramatic alterations in gut flora, supporting such observations made above¹⁵.

15 There is accumulating evidence that bacterial, bowel-derived substances or perhaps "toxins" can be powerful neurotrophic substances with marked clinical effects. *Clostridium botulinum* toxin is a classical example of a microorganism-derived substance which can alter the circulation and cause systemic toxicity resulting in neuromuscular effects which in infants translate clinically as "chronic fatigue" to the point of apnoea and
20 death in extreme cases. The bowels in these children are often markedly constipated. More recently greater recognition of bowel-derived toxins has appeared and there is compelling evidence that certain strains of *Campylobacter jejuni* produce substances which can influence areas of spinal cord and initiate the Guillain-Barré syndrome²¹. Another condition emerging as bowel-flora related is "sudden infant death syndrome" or
25 SIDS. A significantly higher proportion of toxigenic microorganisms and their toxins were found in foetal samples of SIDS babies than in samples from the comparative group of living age-matched babies²². The effects of the toxins were studied in a rabbit model showing that these can profoundly affect the heart rate, blood pressure, respiration and thoracic expansion, and at higher concentrations resulted in prolonged apnoea causing
30 sudden death without distress²³.

- 5 -

In adults, in association with what is called "Irritable Bowel Syndrome", a condition now thought to be caused by abnormalities of the bowel flora, a significant percentage of patients suffer from a sleeping disorder generally termed "sleep apnoea"^{24,25}. Whether the mechanism of this episodic apnoea is bowel toxin mediated via central nervous system pathways akin to that proposed for SIDS or is a pharyngo-laryngeal specific partial paralysis akin to the botulinum toxin or Guillan-Barré region-specific neurotoxicity, is yet unclear.

Australian Patent No. 640349 demonstrates that bowel flora therapy can be used to treat Irritable Bowel Syndrome. That Australian patent describes the use of cultured microorganisms replacement to treat Irritable Bowel Syndrome. It has also been proposed in European Patent No. 245529 and United States Patent No. 4,698,330 that isolated natural or synthetic muramyl peptides are active in mammals as slow-wave sleep inducers. However, United States Patent No. 4,698,330 acknowledges the problem of pyrogenicity which is associated with administration of muramyl peptides for sleep induction, and this document in fact advocates the use of an anti-pyretic agent in conjunction with the muramyl peptide administration. Clearly, the use of anti-pyretic agents is not desirable as the administration of such agents may result in other undesirable side effects.

While it is known, as demonstrated above, to use bowel flora therapy as a treatment for irritable bowel syndrome and it is known to treat sleep disorders with muramyl peptides, there is a need for an effective treatment/prophylactic therapy to be developed for sleep disorders, neuropsychiatric disorders, SIDS, Chronic Fatigue Syndrome and/or childrens *Cl. Botulinum* poisoning, which does not involve the pyrogenicity problems associated with administration of natural isolated or synthetic muramyl peptides.

It is therefore an object of the present invention to develop therapeutic compositions and methods of treatment and/or prophylaxis for sleep disorders, or for inducing sleep or for the treatment and/or prophylaxis of neuropsychiatric disorders, SIDS or Chronic Fatigue Syndrome which will not result in undesirable pyrogenetic side effects. Other objects

of the present invention will become apparent from the following description thereof.

The present inventors have surprisingly found that patients being treated for Irritable Bowel Syndrome (and who had a concomitant diagnosis of Chro: Fatigue Syndrome or insomnia) by bowel flora therapy showed a dramatic disappearance in their sleep disorder.

It has also been shown in other studies by the present inventors that administration of live or dead microorganisms or cell wall containing fragments of microorganisms can be effective in the treatment and/or prophylaxis of sleep disorders or for inducing sleep or for the treatment and/or prophylaxis of neuropsychiatric disorders, SIDS, or Chronic Fatigue Syndrome without resulting in the pyrogenicity problems associated with administration of isolated muramyl peptides.

15 Summary of the Invention

According to a first embodiment of the present invention there is provided a method for the treatment and/or prophylaxis of a sleep disorder or for inducing sleep or for the treatment and/or prophylaxis of a neuropsychiatric disorder or for the treatment and/or prophylaxis of SIDS or Chronic Fatigue Syndrome in a mammal which comprises administering to said mammal an effective amount of whole live or dead enteric microorganisms or cell wall containing fragments of said enteric microorganisms.

According to another embodiment of the present invention there is provided a pharmaceutical composition which comprises whole dead microorganisms or cell wall containing fragments of said microorganisms in association with one or more pharmaceutically acceptable carriers and/or diluents and optionally in association with other pharmaceutically active agents.

According to a further embodiment of the present invention there is provided a method
30 of manufacture of a pharmaceutical composition comprising obtaining a sample of enteric
microorganisms and disrupting said microorganisms and then extracting from disrupted

microorganisms cell wall containing fragments thereof which are then combined with one or more pharmaceutically acceptable carriers, excipients and/or adjuvents.

Detailed Description of the Invention

- 5 Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other elements or integer or group of elements or integers.
- 10 Generally the microorganisms comprehended by the present invention are selected from *Bacteroides*, *Bifidobacterium*, *Eubacteria*, *Fusobacteria*, *Propionibacteria*, *Lactobacilli*, anaerobic cocci, *Ruminococcus*, *Escherichia*, *Gemmiger*, *Clostridium* or *Desulfomonas* genera or species.
- 15 More specifically the microorganisms are selected from *Bacteroides fragilis* ss. *vulgatis*, *Eubacterium aerofaciens*, *Bacteroides fragilis* ss. *thetaiotaomicron*, *Peptostreptococcus productus* II, *Bacteroides fragilis* ss. *distasonis*, *Fusobacterium prausnitzii*, *Coprococcus eutactus*, *Eubacterium aerofaciens* III, *Peptostreptococcus productus* I, *Ruminococcus bromii*, *Bifidobacterium adolescentis*, *Gemmiger formicilis*, *Bifidobacterium longum*,
- 20 *Eubacterium siraeum*, *Ruminococcus torques*, *Eubacterium rectale* III-H, *Eubacterium rectale* IV, *Eubacterium eligens*, *Bacteroides eggerthii*, *Clostridium leptum*, *Bacteroides fragilis* ss. *a*, *Eubacterium bifforme*, *Bifidobacterium infantis*, *Eubacterium rectale* III-F, *Coprococcus comes*, *Bacteroides capillosus*, *Ruminococcus albus*, *Eubacterium formicigenerans*, *Eubacterium hallii*, *Eubacterium ventriosum* I, *Fusobacterium russii*,
- 25 *Ruminococcus obeum*, *Eubacterium rectale* II, *Clostridium ramosum* I, *Lactobacillus leichmanii*, *Ruminococcus callidus*, *Butyrivibrio crossotus*, *Acidaminococcus fermentans*, *Eubacterium ventriosum*, *Bacteroides* AR, *Bacteroides fragilis*, ss. *fragilis*, *Coprococcus catus*, *Eubacterium hadrum*, *E. cylindroides*, *E. ruminantium*, *Eubacterium* CH-I, *Staphylococcus epidermidis*, *Peptostreptococcus* BL, *Eubacterium limosum*, *Bacteroides*
- 30 *praeacutus*, *Bacteroides*, L, *Fusobacterium mortiferum* I, *F. naviforme*, *Clostridium innocum*, *C. ramosum*, *Propionibacterium acnes*, *Ruminococcus flavefaciens*,

- 8 -

Ruminococcus AT, *Peptococcus AU-1*, *Eubacterium AG*, -AK, -AL, -AL1, -AN; *Bacteroides fragilis ss. ovatus*, -ss. d, -ss. j; *Bacteroides L-1*, L-5; *Fusobacterium nucleatum*, *F. mortiferum*, *Escherichia coli*, *Streptococcus morbillorum*, *Peptococcus magnus*, *Peptococcus G*, -AU-1; *Streptococcus intermedius*, *Ruminococcus lactaris*,
 5 *Ruminococcus CO*, *Gemmiger X*, *Coprococcus BH*, -CC; *Eubacterium tenue*, *Eubacterium ramulus*, *Eubacterium AE*, -AG-H, -AG-M, -AJ, -BW-1, *Bacteroides clostridiiformis ss. clostridiiformis*, *B. coagulans*, *B. oralis*, *B. ruminicola ss. brevis*, -ss. *ruminocoli*, *Bacteroides splanchnicus*, *Desulfomonas pigra*, *Bacteroides L-4*, -W-1; *Fusobacterium H*, *Lactobacillus G G*, or *Succinivibrio A*.

10

In a preferred form of the invention, the microorganisms used are a mixture of "Bacteroides and E. coli". The microorganisms may be prepared as a liquid culture or they may be freeze-dried. Microorganisms used in the invention may be live or dead and in fact it is possible to also carry out the invention by utilising cell wall containing
 15 fragments of the dead microorganisms. If the microorganisms are dead they are preferably encapsulated prior to use.

Prior to administering the microorganisms into a mammal, the mammal's existing enteric microflora may be substantially removed. This is however merely an optional aspect of
 20 the invention and is most preferable in the situation where live microorganisms are being administered to the mammal. Preferably the removal of existing enteric microflora is effected by lavage of the gastro-intestinal tract. This can be effected by methods known to those skilled in the art such as ingestion of lavage solutions such as orthostatic salt and polyethyleneglycol solution, enemas or small bowel intubation and lavage. A short
 25 course of antibiotics may be required to rid tissue-invasive pathogens originating in the bowel lumen.

Generally the live or dead microorganisms or cell wall containing fragments thereof are introduced into the gastrointestinal system by enemas or colonoscope, via intubation of
 30 the small bowel using for example a large bore catheter equipped with distal balloon to effect rapid passage down the jejunum, or by infusion into the small bowel, or via the

oral route with a capsule or tablet which may or may not be enterically coated. Preferably the product is administered orally as a capsule or tablet which may be enterically coated or mixed with food or beverage. Most preferably, the product is in a dried powder form which can be mixed with a drink for administration to a patient.

5

The methods of the present invention are applicable to mammals in general, and in particular to humans.

Examples of sleep disorders treated or cured by the methods of the present invention are narcolepsy, hypersomnia, insomnia or sleep apnoea. Sleep disorders comprehended by the present invention may also be caused by immune system depression within the mammal.

Examples of neuropsychiatric disorders which may be treated or prevented by the methods according to the present invention are depression, psychosis, neurosis, catatonia, hyperactivity syndrome, manic depressive illness or anorexia nervosa. It is also envisaged that Chronic Fatigue Syndrome and children's *Cl. Botulinum* poisoning can be treated according to the methods of the present invention. The present invention is also effective as a treatment and/or preventative measure in relation to sudden infant death syndrome (SIDS). The methods of the present invention may further comprise the administration of an adjuvant or other pharmaceutically acceptable carriers or excipients or in fact other pharmaceutically active agents in conjunction with the microorganisms or cell wall containing fragments thereof. Examples of an adjuvant are a gastric suppressant such as a milk product or an antacid which can be used to dampen bacterial inactivation in the stomach, an H₂-receptor antagonist or omeprazole which can be used to suppress stomach acid secretion or a proton pump inhibitor which will have a similar stomach acid secretion suppressive effect.

In general the bowel flora replacement therapy which can be utilised in the present invention is comprehended within Australian Patent No. 640349. In addition, treatment in some instances will also be possible without prior bowel flora removal, simply by

- 10 -

overseeding with the new introduced microorganisms, by longer term ingestion of the new flora utilising enteric-coated capsules. As referred to above gastric acid suppression by the use of milk products, antacids, H²-receptor antagonists or proton pump inhibitors may be utilised to enhance microorganisms survival rates during passage of
5 microorganism through the stomach.

In the case of SIDS, flora manipulation can be employed either as a therapy in suspected pre-SIDS or near-SIDS cases as a treatment of children who on stool screen were found to have detectable toxins of microorganisms associated with SIDS (eg. *Cl. Perfringens*,
10 *Cl.difficile*, *S. Aureaus*, *Cl. botulinum*) or as a broad-spectrum prophylactic therapy in most newborn children to prevent colonisation by toxin-producing microorganisms.

Although not wishing to be bound by theory it appears likely that the therapeutic benefits associated with the present invention are due to liberation of muramyl peptides from the
15 microorganisms administered according to the invention. It appears however, that the muramyl peptides can only be liberated by the action of the enzymes N-acetylmuramoyl-L-alanine-amidase from bacterial cell walls of bacterial cell fragments. In turn therefore, it appears most likely that the therapeutic benefit associated with the present invention is achieved after microorganisms which may have been administered alive or dead but
20 whole have been broken down into cell wall containing fragments. As a result, the method of increasing muramyl peptide availability within the body and thereby increasing the factor S levels in the brain to induce sleep and improved immune system activity can be achieved by administering either whole live or dead microorganisms or cell wall containing fragments of the microorganisms which contain muramyl peptides within their
25 cell walls. Surprisingly, the methods according to the invention overcome the pyrogenicity problems associated with administering isolated natural muramyl peptides or synthetic muramyl peptides.

The cell wall containing fragments of the microorganisms of the invention can be
30 obtained from any of the microorganism species referred to herein and especially species from the Genera *Escherichia*, *Acidophilus* and *Bifidobacterium* by lysing or disrupting

the cells in any of a number of ways. For example, the cells may be subjected to pulverisation by passage through a blender or milling with glass beads. They may be centrifuged, sonicated, homogenised, heated, exposed to osmotic shock or treated with enzymes or chemicals which result in cell lysis or disruptions. In general it is suggested
5 that during the disruption process the cells be maintained in solution although it is also possible to effect disruption when the cells are not in solution.

An example of enzymes to effect cell disruption is the use of enzymes of the lysozyme family. Lysozymes are bacteriacidal enzymes which cleave the β -1,4 bond of the
10 bacterial cell wall and thereby liberate the cell contents. Preferably lysozyme treatment is carried out in a sucrose solution so that the products are cleaved bacterial cell walls and spheroplasts. By carrying this process out in the presence of sucrose the spheroplasts will be prevented from lyzing and therefore the process of isolating the cell wall material from the spheroplast will be simplified as it is then not necessary to
15 separate the cell wall material from all other intracellular material. This separation can be carried out in a number of ways including for example dialysis or centrifugation followed by filtration.

The formation of pharmaceutical compositions comprising the microorganisms or cell
20 wall containing fragments thereof can follow the standard procedures generally known in the art, and reference can conveniently be made to Wellington's Pharmaceutical Sciences, 17th Edition, Mack Publishing Co., Easton, Pennsylvania, United States of America.

25 The dosage regime will depend upon many factors such as for example the age, sex, weight and the particular species of mammal concerned. Also of importance in considering the required administration level will be the nature of the disorder which is intended to be treated or prevented according to the method of the present invention, also taking into account any other therapeutic treatments which the patient concerned may be
30 concurrently undergoing. For example, from about 0.5 micrograms to about 5 grams per kilogram body weight of microorganism or cell wall containing fragments thereof

- 12 -

may be administered to the patient per day. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. The active compound may be administered in a convenient manner such as by the oral route or by infusion or intubation into the intestines. Depending upon the route of administration, the microorganism or cell wall containing fragments thereof may be required to be coated in a material to protect them from the action of enzymes, acids and other natural conditions which may inactivate them. For example, and as referred previously the peptides may be administered in an adjuvant or co-administered with enzyme inhibitors or even within lysozymes or standard pharmaceutical capsules. Adjuvants according to the invention have been referred to above and enzyme inhibitors include for example pancreatic trypsin inhibitor while lysozyme may include water-in-oil-in-water CGF emulsions as well as conventional lysozyme. When the microorganism cell wall containing fragments thereof are suitable protected as described above they may be orally administered for example with an inert diluent or with an assimilable edible carrier, or may be enclosed in hard or soft shell gelatin capsule, may be compressed into tablets or may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, capsules, elixirs suspensions, syrups, wafers and the like. It is preferred that such compositions and preparations should contain at least one percent by weight of the active material.

EXAMPLES

25

Example 1

A 49 year old female patient with longstanding insomnia and abdominal symptoms of Irritable Bowel Syndrome, with marked food intolerance underwent antibiotic pre-treatment, orthostatic bowel lavage and oral colon administration of a mixture of 20 anaerobic and aerobic bacteria over 2 days. Within 5 days the food intolerance and Irritable Bowel Syndrome symptoms were no longer present. The patient could now eat foods which previously caused marked abdominal cramping and bloating. Most

- 13 -

significantly, insomnia disappeared and she obtained restful sleep with ease of sleep and no early morning waking.

Example 2

5

A 50 year old male with severe diarrhoea negative to all investigations, was also suffering with proven sleep apnoea and chronic fatigue. He was using standard sleep apnoea nocturnal positive pressure ventilation (CPAP) with poor clinical response. His tiredness was quite profound. Following bowel flora change (as in Example 1) his
10 diarrhoea abated, resulting in formed stools. Significantly his fatigue left him and he no longer relied upon the ventilating machine at night. Furthermore, lunchtime somnolence (had to sleep in his car during lunchtime) disappeared and his mental state was no longer described as "fuzzy".

15

Example 3

In a 9 year old child with chronic constipation, undiagnosed moderate failure to thrive in spite of multiple investigations and unexplained anorexia - leading to the diagnosis of "anorexia nervosa", bowel flora treatment was carried out. Pre-treatment with
20 vancomycin and other antibiotics, was followed by oral and injections of a mixture of cultured enteric bacteria. Prompt reversal of anorexia took place while the patient was begun on the antibiotics, and continued long-term following bacterial therapy.

It is to be recognised that the present invention has been described by way of example
25 only, and that modifications and/or alterations which would be obvious to a person skilled in the art based on the disclosure herein are also considered to be included within the scope of the present invention as defined by the appended claims.

1. Fencel V, Koski G, Pappenheimer JR., *Factors in cerebrospinal fluid from goats that affect sleep activity in rats*. American J Physiology 1971; 216: 565-589.
2. Monnier M, Hosli L., *Dialysis of sleep and waking factors in blood of the rabbit*. Science 1964; 123: 796-798.
3. Krueger JM, Bascik J, Garcia-Arras J., *Sleep-promoting material from human urine and its relation to factor S from brain*. American J Physiol 1980; 238: E116-E123.
4. Krueger JM., *Somnogenic activity of muramyl peptides*. Trends in Pharmacological Sciences 1985; 6:218-221.
5. Martin SA, Karnovsky ML, Kreuger JL, Pappenheimer JR, Biemann K., *Peptidoglycans as promoters of slow-wave sleep*. The Journal of Biological Chemistry 1984; 259:12652-12658.
6. Davis BD, Dulbecco R, Eisen HN, Ginsberg HS, Wood WB, McCarty M., *Microbiology*, 2nd ed., Harper and Row, New York, 1973.
7. Pappenheimer JR., *Induction of sleep by muramyl peptides*. The Journal of Physiology 1983; 336:1-11.
8. Kreuger JM, Walter J, Levin C., *Factor S and related somnogens: an immune theory for slow wave sleep*. In 'Brain Mechanisms of Sleep' eds McGinty DJ, Drucker-Colin R, Morrison A, Parmeggiani PL, Raven Press, New York 1985, pp253-275.
9. Brown R, Price RJ, King MG, Husband AJ., *Autochthonous Intestinal Bacteria and Coprophagy: A possible Contribution to the Ontogeny and Rhythmicity of Slow Wave Sleep in Mammals*. Medical Hypotheses 1988; 26:171-175.

10. Rotimi VO, Duerden BL., *The development of the bacterial flora in normal neonates*. Journal of Medical Microbiology 1981; 14:51-62.
- 5 11. Brunel A, Gouet P., *Kinetic bacterial implantation in the intestine of newborn rats*. In "Recent Advances in Germfree Research" eds. Sasaki S, Ozawa A, Hashimoto K. Tokai University Press, Tokyo, 1981, pp 185-188.
- 10 12. Jouvett-Mounier D, Astic L, Lactote D., *Ontogenesis of the states of sleep in rat, cat, and guinea pig during the first postnatal month*. Developmental Psychobiology 1969; 2:216-239.
13. Foo MC, Lee A., *Immunological response of mice to members of the autochthonous intestinal flora*. Infection and Immunity 1972; 6:525-532.
- 15 14. Salzarulo P, Fagioli I, Salamon F, Ricour C., *Developmental trend of quiet sleep is altered by early human malnutrition and recovered by nutritional rehabilitation*. Early Human Development 1982; 7:257-264.
- 20 15. Dubos RJ, Schaedler RW., *The effect of diet on the faecal bacterial flora of mice and their resistance to infection*. Journal of Experimental Medicine 1962; 115:1161.
- 25 16. Smith W., *The development of the flora of the alimentary tract in young animals*. Journal of Pathology and Bacteriology 1965; 90: 495-413.
17. Davenne D, Krueger J., *Muramyl dipeptide promotes quiet sleep in rabbit neonate*. Neuroscience Letters 1986; 26:S132.
- 30 18. Brown R, Price RJ, King MG, Husband AJ., *Are antibiotic effects on sleep behaviour in the rat due to modulation of gut bacteria?* Physiology and Behaviour 1990; 48:561-565.

- 16 -

19. Rhee Y-H, Kim H-1., *The correlation between sleeping-time and numerical change of intestinal flora in psychiatric insomnia patients.* Bull Nat Sci Chungbuk Natl Univ 1987; 1: 159-172.
- 5 20. Tvede M. Rask-Madsen J., *Bacteriotherapy for chronic relapsing Clostridium difficile diarrhoea in six patients.* Lancet 1989; i: 1156-1160.
21. Mishu B, *et al.*, Annals of Internal Medicina 1993; 118-947.
- 10 22. Murrell WG, Stewart BJ, O'Neill C, Siarakas-S, Kariks S., *Enterotoxigenic bacteria in the sudden infant death syndrome.* J Med Microbiol 1993; 39: 114-127.
- 15 23. Sirakas S, Dumas E, Murrell WG., *Effect of bacterial toxins on the rabbit, a possible animal model for SIDS.* In: Walker A, McMillen C, Finch C, editors, Proceedings of the Second SIDS Family International Conference; 1992, Feb 12-16; Sudney. Ithaca, NY: Perinatology Press, 1993.
- 20 24. Kumar D, Thompson PD, Wingate DL, *et al.*, *Abnormal REM sleep in the Irritable Bowel Syndrome.* Gastroenterology 1992; 103: 12-17.
25. Orr WC., *The Irritable Bowel Syndrome: In your Dreams?* Amer J Gastroenterol 1993; 88: 781-783.

CLAIMS

1. A method for the treatment and/or prophylaxis of a sleep disorder or of inducing sleep or for the treatment and/or prophylaxis of a neuropsychiatric disorder or for the treatment and/or prophylaxis of SIDS or for the treatment and/or prophylaxis of Chronic Fatigue Syndrome or children's Cl. botulinum poisoning in a mammal, comprising administering to said mammal an effective amount of whole live or dead enteric microorganisms or cell wall containing fragments of said microorganisms.
2. The method of claim 1, wherein said microorganisms are selected from *Bacteroides*, *Bifidobacterium*, *Eubacteria*, *Fusobacteria*, *Propionibacteria*, *Lactobacilli*, anerobic cocci, *Ruminococcus*, *Escherichia*, *Gemmiger*, *Clostridium* or *Desulfomonas* genera or species.
3. The method of claim 2, wherein said microorganisms are selected from *Bacteroides fragilis* ss. *vulgatis*, *Eubacterium aerofaciens*, *Bacteroides fragilis* ss. *thetaiotaomicron*, *Peptostreptococcus productus* II, *Bacteroides fragilis* ss. *distasonis*, *Fusobacterium prausnitzii*, *Coprococcus eutactus*, *Eubacterium aerofaciens* III, *Peptostreptococcus products* I, *Ruminococcus bromii*, *Bifidobacterium adolescentis*, *Gemmiger formicilis*, *Bifidobacterium longum*, *Eubacterium siraeum*, *Ruminococcus torques*, *Eubacterium rectale* III-H, *Eubacterium rectale* IV, *Eubacterium eligens*, *Bacteroides eggerthii*, *Clostridium leptum*, *Bacteroides fragilis* ss. *a*, *Eubacterium biforme*, *Bifidobacterium infantis*, *Eubacterium rectale* III-F, *Coprococcus comes*, *Bacteroides capillosus*, *Ruminococcus albus*, *Eubacterium formicigenerans*, *Eubacterium hallii*, *Eubacterium ventriosum* I, *Fusobacterium russii*, *Ruminococcus obeum*, *Eubacterium rectale* II, *Clostridium ramosum* I, *Lactobacillus leichmanii*, *Ruminococcus callidus*, *Butyrivibrio crossotus*, *Acidaminococcus fermentans*, *Eubacterium ventriosum*, *Bacteroides* AR, *Bacteroides fragilis*, ss. *fragilis*, *Coprococcus catus*, *Eubacterium hadrum*, *E. cylindroides*, *E. ruminantium*, *Eubacterium* CH-1, *Staphylococcus epidermidis*, *Peptostreptococcus* BL,

- Eubacterium limosum*, *Bacteroides praeacutus*, *Bacteroides*, *L.*, *Fusobacterium mortiferum* 1, *F. naviforme*, *Clostridium innocum*, *C. ramosum*, *Propionibacterium acnes*, *Ruminococcus flavefaciens*, *Ruminococcus AT*, *Peptococcus AU-1*, *Eubacterium AG*, -AK, -AL, -AL1, -AN; *Bacteroides fragilis*
 5 *ss. ovatus*, -*ss. d*, -*ss. f*; *Bacteroides L-1*, *L-5*; *Fusobacterium nucleatum*, *F. mortiferum*, *Escherichia coli*, *Streptococcus morbillorum*, *Peptococcus magnus*, *Peptococcus G*, -AU-1; *Streptococcus intermedius*, *Ruminococcus lactaris*, *Ruminococcus CO*, *Gemmiger X*, *Coprococcus BH*, -CC; *Eubacterium tenue*, *Eubacterium ramulus*, *Eubacterium AE*, -AG-H, -AG-M, -AJ, -BW-1, *Bacteroides*
 10 *clostridiiformis ss. clostridiiformis*, *B. coagulans*, *B. oralis*, *B. ruminicola ss. brevis*, -*ss. ruminocoli*, *Bacteroides splanchnicus*, *Desulfomonas pigra*, *Bacteroides L-4*, -W-1; *Fusobacterium H*, *Lactobacillus G*, or *Succinivibrio A*.
4. The method of claim 3, wherein said microorganisms are a mixture of
 15 *Bacteroides* and *E. coli*.
5. The method of any one of claims 1 to 4, wherein said microorganisms are in a liquid culture or are freeze dried.
- 20 6. The method of any one of claims 1 to 5, wherein said microorganisms are live.
7. The method of any one of claims 1 to 5, wherein said microorganisms are dead.
8. The method of claim 7, wherein said microorganisms are sonicated and
 25 encapsulated.
9. The method of claim 7 wherein cell wall containing fragments of said microorganisms are administered.
- 30 10. The method of any one of claims 1 to 9, wherein said microorganisms or cell wall containing fragments thereof are administered by colonoscopic infusion, by

enema, by infusion into the small bowel via an endoscope, by intubation, or by ingestion.

11. The method of any one of claims 1 to 10, further comprising substantially removing the mammal's existing enteric microflora prior to administering said microorganisms or cell wall containing fragments thereof.
12. The method of claim 11, wherein said mammal's existing microflora is removed by administering an antibiotic and/or by bowel lavage.
13. The method of any one of claims 1 to 12, wherein said sleep disorder is narcolepsy, hypersomnia, insomnia or sleep apnoea.
14. The method of any one of claims 1 to 13, wherein said neuropsychiatric disorder is depression, psychosis, neurosis, catatonia, hyperactivity syndrome, manic depressive illness or anorexia nervosa.
15. The method of any one of claims 1 to 14, for treatment or prophylaxis of chronic fatigue syndrome or children's *Cl. botulinum* poisoning.
16. A pharmaceutical composition comprising whole dead microorganisms or cell wall containing fragments thereof in association with one or more pharmaceutically acceptable carriers, excipients, and/or adjuvants.
17. The composition of claim 16, wherein said microorganisms are selected from *Bacteroides fragilis ss. vulgatis*, *Eubacterium aerofaciens*, *Bacteroides fragilis ss. distasonis*, *Fusobacterium prausnitzii*, *Coprococcus eutactus*, *Eubacterium aerofaciens III*, *Peptostreptococcus products I*, *Ruminococcus bromii*, *Bifidobacterium adolescentis*, *Gemmiger formicilis*, *Bifidobacterium longum*, *Eubacterium siraeum*, *Ruminococcus torques*, *Eubacterium rectale III-H*, *Eubacterium rectale IV*, *Eubacterium eligens*, *Bacteroides eggerthii*, *Clostridium*

- 20 -

leptum, *Bacteroides fragilis* ss. *a*, *Eubacterium bifforme*, *Bifidobacterium infantis*,
Eubacterium rectale III-F, *Coprococcus comes*, *Bacteroides capillosus*,
Ruminococcus albus, *Eubacterium formicigenerans*, *Eubacterium hallii*,
Eubacterium ventriosum I, *Fusobacterium russii*, *Ruminococcus obeum*,
5 *Eubacterium rectale* II, *Clostridium ramosum* I, *Lactobacillus leichmanii*,
Ruminococcus callidus, *Butyrivibrio crossotus*, *Acidaminococcus fermentans*,
Eubacterium ventriosum, *Bacteroides* AR, *Bacteroides fragilis*, ss. *fragilis*,
Coprococcus catus, *Eubacterium hadrum*, *E. cylindroides*, *E. ruminantium*,
Eubacterium CH-1, *Staphylococcus epidermidis*, *Peptostreptococcus* BL,
10 *Eubacterium limosum*, *Bacteroides praeacutus*, *Bacteroides*, L, *Fusobacterium*
mortiferum I, *F. naviforme*, *Clostridium innocum*, *C. ramosum*,
Propionibacterium acnes, *Ruminococcus flavefaciens*, *Ruminococcus* AT,
Peptococcus AU-1, *Eubacterium* AG, -AK, -AL, -AL1, -AN; *Bacteroides fragilis*
ss. *ovatus*, -ss. *d*, -ss. *f*; *Bacteroides* L-1, L-5; *Fusobacterium nucleatum*, *F.*
15 *mortiferum*, *Escherichia coli*, *Streptococcus morbillorum*, *Peptococcus magnus*,
Peptococcus G, -AU-1; *Streptococcus intermedius*, *Ruminococcus lactaris*,
Ruminococcus CO, *Gemmiger* X, *Coprococcus* BH, -CC; *Eubacterium tenue*,
Eubacterium ramulus, *Eubacterium* AE, -AG-H, -AG-M, -AJ, -BW-1, *Bacteroides*
clostridiiformis ss. *clostridiiformis*, *B. coagulans*, *B. oralis*, *B. ruminicola* ss.
20 *brevis*, -ss. *ruminocoli*, *Bacteroides splanchnicus*, *Desulfomonas pigra*,
Bacteroides L-4, -W-1; *Fusobacterium* H, *Lactobacillus* G, or *Succinivibrio* A.

18. The composition of claim 17, wherein said microorganisms are a mixture of
Bacteroides and *E. coli*.
- 25 19. The composition of any one of claims 16 to 18 in an oral dosage form.
20. The composition of claim 19 in a form suitable to be administered by
colonoscopic infusion, enema, infusion into the small bowel via an endoscope or
30 by intubation.

21. The composition of any one of claims 16 to 20 wherein said adjuvants are one or more of gastric suppressants, H₂-receptor antagonists or proton pump inhibitors.
- 5 22. A method of manufacture of a pharmaceutical composition comprising obtaining a sample of enteric microorganisms and disrupting said microorganisms and then extracting from disrupted microorganisms cell wall containing fragments thereof which are then combined with one or more pharmaceutically acceptable carriers, excipients and/or adjuvants or optionally other pharmaceutically active agents.
- 10 23. The method of claim 22 wherein said microorganisms are selected from *Bacteroides fragilis ss. vulgatis*, *Eubacterium aerofaciens*, *Bacteroides fragilis ss. vulgatis*, *Eubacterium aerofaciens*, *Bacteroides fragilis ss. thetaiotaomicron*, *Peptostreptococcus productus II*, *Bacteroides fragilis ss. distasonis*,
 15 *Fusobacterium prausnitzii*, *Coprococcus eutactus*, *Eubacterium aerofaciens III*, *Peptostreptococcus products I*, *Ruminococcus bromii*, *Bifidobacterium adolescentis*, *Gemmigerformicilis*, *Bifidobacterium longum*, *Eubacterium siraeum*, *Ruminococcus torques*, *Eubacterium rectale III-H*, *Eubacterium rectale IV*, *Eubacterium eligens*, *Bacteroides eggerthii*, *Clostridium leptum*, *Bacteroides fragilis ss. a*, *Eubacterium bifforme*, *Bifidobacterium infantis*, *Eubacterium rectale III-F*, *Coprococcus comes*, *Bacteroides capillosus*, *Ruminococcus albus*, *Eubacterium formicigenerans*, *Eubacterium hallii*, *Eubacterium ventriosum I*, *Fusobacterium russii*, *Ruminococcus obeum*, *Eubacterium rectale II*, *Clostridium ramosum I*, *Lactobacillus leichmanii*, *Ruminococcus callidus*, *Butyrivibrio crossotus*, *Acidaminococcus fermentans*, *Eubacterium ventriosum*, *Bacteroides AR*, *Bacteroides fragilis, ss. fragilis*, *Coprococcus catus*, *Eubacterium hadrum*, *E. cylindroides*, *E. ruminantium*, *Eubacterium CH-1*, *Staphylococcus epidermidis*, *Peptostreptococcus BL*, *Eubacterium limosum*, *Bacteroides praeacutus*, *Bacteroides, L*, *Fusobacterium mortiferum I*, *F. naviforme*, *Clostridium innocum*,
 20 *C. ramosum*, *Propionibacterium acnes*, *Ruminococcus flavefaciens*, *Ruminococcus AT*, *Peptococcus AU-1*, *Eubacterium AG*, *-AK*, *-AL*, *-AL1*, *-AN*; *Bacteroides*
- 25
 30

- 22 -

- 5 *fragilis* ss. *ovatus*, -ss. *d*, -ss. *f*; *Bacteroides* L-1, L-5; *Fusobacterium nucleatum*, *F. mortiferum*, *Escherichia coli*, *Streptococcus morbillorum*, *Peptococcus magnus*, *Peptococcus G*, -AU-1; *Streptococcus intermedius*, *Ruminococcus lactaris*, *Ruminococcus CO*, *Gemmiger X*, *Coprococcus BH*, -CC; *Eubacterium tenue*,
 10 *Eubacterium ramulus*, *Eubacterium AE*, -AG-H, -AG-M, -AJ, -BW-1, *Bacteroides clostridiiformis* ss. *clostridiiformis*, *B. coagulans*, *B. oralis*, *B. ruminicola* ss. *brevis*, -ss. *ruminocoli*, *Bacteroides splanchnicus*, *Desulfomonas pigra*, *Bacteroides* L-4, -W-1; *Fusobacterium H*, *Lactobacillus G*, or *Succinivibrio A*.
- 10 25. The method of claim 24 wherein said microorganisms are a mixture of *Bacteroides* and *E. coli*.
26. The method of any one of claims 22 to 25 wherein disruption is by sonication, dehydration, centrifugation, pulverisation, heating, osmotic shock,
 1 homogenisation, milling with glass beads or treatment with enzymes.
27. The method of claim 26 wherein said enzyme is lysozyme.
28. The method of any one of claims 22 to 27 wherein said extracting is by
 20 centrifugation, filtration or dialysis.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 95/00664

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl⁶: A61K 035/74, 035/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC : A61K 035/74 and keywords below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU : IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Derwent Database, file WPAT; Chemical Abstracts Service; file CASM; Medline.

Keywords : gastrointestinal, enteric, microbiol, microorganism, muramyl, flora, microflora, bacteria, sleep, insomnia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU 40617/89 A1 (THOMAS JULIUS BORODY) 5 March 1990 (See whole document)	1-28
X	Journal of Rheumatology 1989; (Supplement 19), Vol 16. Krueger J.M. <i>et al</i> "Bacterial Products, Cytokines and Sleep". (See whole document)	1-28
A	Am. J. Physiol. Vol. 260, No. 1, Pt. 2, January 1991. Johannsen L <i>et al</i> "Macrophages produce somnogenic and pyrogenic muramyl peptides during digestion of staphylococci".	1-28

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
16 January 1996

Date of mailing of the international search report
22 JAN 1996

Name and mailing address of the ISA/AU
AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION
PO BOX 200
WODEN ACT 2606
AUSTRALIA Facsimile No.: (06) 285 3929

Authorized officer

BERNARD NUTT

Telephone No.: (06) 283 2491

International Application No.

Information on patent family members

PCT/AU 95/00664

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
AU	89/40617	CA WO	1333564 9001335	EP	433299	US	5443826
END OF ANNEX							